CLAIMS

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows. We claim:

1. An amplification probe adapted to permit enhanced detectable labelling of a selected nucleic acid target, such probe comprising at least two regions of nucleic acid sequences: a first region including a sequence complementary to a sequence on a selected primary probe which also contains a sequence complementary to a sequence of said selected nucleic acid target, and a second region including a plurality of discretely labellable sequence units.

2. An amplification probe as claimed in Claim 1, wherein the number of discretely labelable sequence units ranges from two to fifty in number.

3. An amplification probe as claimed in Claim 1, wherein each said discretely labelable sequence unit comprises a sequence of nucleotide bases hybridizable to a complementary sequence on a labelling probe, said labelling probe covalently attached to a detectable chemical label.

4. An amplification probe as claimed in Claim 3, wherein the length of each sequence unit ranges from 16 to 100 nucleotides.

5. A method for enhancing detectable labelling of probe-target complexes in nucleic acid hybridization assays incorporating an amplification probe adapted to permit

enhanced detectable labelling of a selected nucleic acid target, such probe comprising at least two regions of nucleic acid sequences: a first region including a sequence complementary to a sequence on a selected primary probe which also contains a sequence complementary to a sequence of said selected nucleic acid target, and a second region including a plurality of discretely labelable sequence units.

6. A method as claimed in Claim 5 wherein each said discretely labelable sequence unit comprises a sequence of nucleotide bases hybridizable to a complementary sequence on a labelling probe, said labelling probe covalently attached to a detectable chemical label.

7. A method as claimed in Claim 6 wherein the detectable chemical label is selected from an enzymatically active group, a fluorescer, a chromophore, a luminescer, a specifically bindable ligand, or a radioisotope.

8. A method as claimed in Claim 5 wherein a label attached to each said discretely labelable sequence units interacts with a reagent member of a label detection system to provide the detectable response.

9. The method as claimed in claim 8 wherein the detectable chemical label is a substrate, cofactor, or inhibitor of an enzyme which is the member of the label detection system with which the label interacts to provide the detectable response.

10. The method as claimed in claim 9 wherein the label is a substrate which is acted on by the enzyme to produce a colorimetric, fluorescent or luminescent signal.

11. The method as claimed in claim 9 wherein the label is a prosthetic group of an enzyme and wherein the apoenzyme of

such enzyme is the member of the label detection system with which the label interacts to produce the catalytically active holoenzyme.

12. The method as claimed in claim 11 wherein the prosthetic group is FAD and the apoenzyme is apo(glucose oxidase).

13. A method for detecting specific nucleic acid sequences comprising:

a) hybridizing a first sequence of a primary polynucleotide probe to a selectable target nucleic acid sequence wherein the primary probe has a means for binding to an amplification probe comprising a nucleic acid sequence adapted to permit enhanced detectable labelling, the amplification probe being capable of hybridizing to at least one labelling probe comprising a nucleic acid sequence conjugated to a chemical label.

b) immobilizing the target-probe complex;

c) exposing the immobilized target-probe complex to said amplification probe, such probe comprising at least two regions of nucleic acid sequences: a first region including a sequence complementary to a sequence on a selected primary probe which also contains a sequence complementary to a sequence of said selected nucleic acid target, and a second region including a plurality of discretely labelable sequence units, under conditions that allows the amplification probe to hybridize to the target-probe complex;

d) exposing the hybridized amplification probe to a labelling probe covalently attached to a detectable chemical label, such probe comprising sequences complementary to sequences on the amplification probe,

under conditions that allows many labelling probes to hybridize to the amplification probe;

e) observing the presence or absence of the detectable chemical label, covalently attached to said labelling probe, in association with the sample as indicating the presence or absence of the target sequence.

14. A method as claimed in Claim 13 wherein the means for immobilizing the probe-target complex involves an antibody reagent capable of binding to DNA/DNA, DNA/RNA or RNA/RNA duplexes formed between the selectable target sequence and the complementary primary probe sequence.

15. A method as claimed in Claim 14 wherein the antibody reagent is:
(i) selective for binding DNA/RNA hybrids wherein one of the probe and the sequence to be detected is DNA and the other is RNA,
(ii) selective for binding RNA/RNA hybrids wherein both the probe and the sequence to be detected are RNA, or
(iii) selective for binding intercalation complexes wherein the duplexes formed in the assay comprise a nucleic acid intercalator bound thereto in the form of intercalation complexes.

16. A method as claimed in Claim 13 wherein the detectable chemical label is selected from an enzymatically active group, a fluorescer, a chromophore, a luminescer, a specifically bindable ligand, or a radioisotope.

17. A method as claimed in Claim 13 wherein a label attached to each said discretely labelable sequence units interacts with a reagent member of a label detection system to provide the detectable response.

18. The method as claimed in claim 17 wherein the label is a substrate, cofactor, or inhibitor of an enzyme which is the member of the label detection system with which the label interacts to provide the detectable response.

19. The method as claimed in claim 18 wherein the label is a substrate which is acted on by the enzyme to produce a colorimetric, fluorescent or luminescent signal.

20. The method as claimed in claim 18 wherein the label is a prosthetic group of an enzyme and wherein the apoenzyme of such enzyme is the member of the label detection system with which the label interacts to produce the catalytically active holoenzyme.

21. The method as claimed in claim 20 wherein the prosthetic group is FAD and the apoenzyme is apo(glucose oxidase).

22. A method as claimed in Claim 13 applied to the detection of a particular nucleic acid sequence in a test medium wherein the test medium comprises a biological sample which has been subjected to conditions to release and denature nucleic acids present therein.

23. A method as claimed in Claim 22 wherein the biological sample includes food substances and the target nucleic acid sequence is of a bacterial microorganism.

24. A method as claimed in Claim 22 wherein the biological sample includes food substances and the target nucleic acid sequence is of a virus.

25. A reagent system for detecting a particular polynucleotide sequence in a test sample, comprising:
(1) a primary nucleic acid probe comprising at least one single stranded base sequence that is substantially complementary to the sequence to be detected,

(ii) an antibody reagent capable of binding to hybrids formed between any of the particular polynucleotide sequences to be detected in the sample and the primary probe, but incapable of binding substantially to single stranded nucleic acids,

(iii) an amplification probe adapted to permit enhanced detectable labelling of a selected nucleic acid target, such probe comprising at least two regions of nucleic acid sequences: a first region including a sequence complementary to a sequence on a selected primary probe which also contains a sequence complementary to a sequence of said selected nucleic acid target, and a second region including a plurality of discretely labelable sequence units,

(iv) a labelling probe covalently attached to a detectable chemical label, such probe comprising sequences complementary to sequences on the amplification probe.

26. The reagent system of claim 25 which additionally comprises a denaturation agent capable fo converting double stranded nucleic acids in a test sample into single stranded form.

27. A diagnostic kit for detecting a particular polynucleotide sequence within a sample comprising:

(i) a primary nucleic acid probe comprising at least one single stranded base sequence that is substantially complementary to the sequence to be detected,

(ii) an antibody reagent capable of binding to hybrids formed between any of the particular polynucleotide sequences to be detected in the sample and the primary probe, but incapable of binding substantially to single stranded nucleic acids,

(iii) an amplification probe adapted to permit enhanced detectable labelling of a selected nucleic acid target, such probe comprising at least two regions of nucleic acid sequences: a first region including a sequence complementary to a sequence on a selected primary probe

which also contains a sequence complementary to a sequence of said selected nucleic acid target, and a second region including a plurality of discretely labelable sequence units,

(iv) a labelling probe covalently attached to a detectable chemical label, such probe comprising sequences complementary to sequences on the amplification probe.

28. The diagnostic kit of claim 27 which additionally comprises a denaturation agent capable fo converting double stranded nucleic acids in a test sample into single stranded form.

29. A diagnositic kit for the detection of E. coli in food comprising the diagnostic kit as claimed in claim 27.